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Influence of the Water-Accommodated Fraction of No. 2 Fuel Oil on Energetics of 'Cancer irroratus' Larvae

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16. ABSTRACT

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Influence of the Water-Accommodated Fraction of No. 2 Fuel Oil on Energetics of *Cancer irroratus* Larvae

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Abstract

The influence of the water-accommodated fraction of No. 2 fuel oil on aspects of larval development of *Cancer irroratus* (Say) has been investigated. Rates of survival, respiration, ingestion and growth were estimated for both oil-treated and untreated larvae. Larvae reared in oil-treated water exhibited lower survival to megalopa than did larvae reared in untreated water. The presence of oil also altered several aspects of larval energetics. Reductions were seen in food consumption and growth rate while the metabolic cost of maintenance increased. One overall effect that may occur as a result of lowered larval survival and growth rate would be the reduction in larval recruitment in oil-polluted areas.

Introduction

Organisms that inhabit estuarine and near-shore coastal waters are often subjected to many pollutants. One such pollutant that has been attracting much attention is oil, largely due to several spectacular losses of oil resulting from the grounding of oil tankers ("Torrey Canyon" in 1967; "Argo Merchant" in 1976; "Amoco Cadiz" in 1978).

Many biological studies of oil have been specifically spill-related and the focus has been on assessing short- and long-term effects of the oil on local marine biota (Smith, 1970; Blumer *et al.*, 1973; Chan, 1973). This work has also been supplemented by determinations of lethal dose levels for organisms in laboratory studies (for a review see Craddock, 1977). There are other sources of oil contamination of the marine environment. Indeed, these could present a more insidious threat to marine organisms than the more dramatic large oil spills. Spillage from shoreline storage areas, discharge of ballast waters by tankers, and discharge of oil via sewerage effluents and storm sewers result in low-level, chronic pollution (Blumer, 1971; Farrington and Quinn, 1973). Recent research on the sublethal effects of oil is relevant to both chronic discharges and long-term release of oil from the benthos after a major spill. Sublethal concentrations of oil are known to affect success of hatching, survival rates, developmental rates and behavior (Anderson *et al.*, 1977; Bigford, 1977; Pechenik *et al.*, 1980; for details of earlier literature see reviews by Moore and Dwyer, 1974; Johnson, 1977; Patten, 1977). Changes in respiratory rate and/or heart-beat rate in invertebrates and estuarine fish, and depression or loss of osmoregulatory ability in several species have also been reported (Avolizi and Nuwayhid, 1974; Anderson *et al.*, 1977).

Little is known about the influence of oil on energy flow through organisms. Changes in energy flow may ultimately alter the dynamics of a population and an ecosystem. We are aware of only a few papers that directly address the question of energy balance. Gilfillan and his co-workers (Gilfillan, 1975; Gilfillan *et al.*, 1976; Gilfillan and Vandermeulen, 1978) have calculated carbon fluxes for several species of bivalves either treated with oil in controlled laboratory experiments or in field studies following an actual oil spill. Net carbon flux in bivalves studied was reduced, indicating an energy imbalance. Eldridge *et al.* (1977) examined influences of benzene on the energetics of embryos and larvae of the Pacific herring *Clupea harengus*. Their findings show that, even with low-level doses of ben-

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zene (0.1 µl l^{-1}), both anabolic and catabolic (embryonic growth rate and yolk utilization rate) processes are disrupted. Edwards (1978) determined the effects of the water-soluble fraction of crude oil on metabolism, growth and the carbon budget of the shrimp *Crangon crangon*. New carbon turnover was reduced, as was the rate of metabolism and growth.

The present study was undertaken to determine effects of the water-accommodated fraction (WAF) of No. 2 fuel oil on components of the energy budgets for larvae of the rock crab *Cancer irroratus*.

Materials and Methods

Test solutions of 0.1 ppm WAF No. 2 fuel oil (Exxon Corporation)[3] were obtained from the flow-through oil-dosing system described by Hyland *et al.* (1977). Total hydrocarbon concentration in both control and 0.1 ppm WAF tanks was determined three times each week using infrared spectrophotometry. During the time period of our experiments, the measured oil concentration in the 0.1 ppm WAF tank ranged from 0.19 to 0.05 ppm WAF ($\bar{x}$ = 0.095 ppm WAF). Water drawn into the glass rearing-jars from this tank was filtered through a small amount of cotton in order to remove large particulate material. This process results in the loss of some of the accommodated fraction (Pechenik *et al.*, 1980). Since actual oil hydrocarbon concentrations in the WAF tank and rearing tanks were not determined on a daily basis, 0.1 ppm should be considered a nominal concentration.

Ovigerous females of *Cancer irroratus* (Say) were collected between December, 1977 and April, 1978 from Narragansett Bay, Rhode Island, USA. Females with undamaged egg masses were brought into the laboratory and maintained in unfiltered Narragansett Bay water (ca. 30‰ S and 15°C).

Newly hatched Stage I zoeae were placed into either 250 ml glass jars (50 larvae jar^{-1}; used to determine survival) or 1 l jars (200 larvae jar^{-1}; used to determine physiological state) containing control or oil-treated seawater. Jars of treated seawater, as well as jars of control seawater, were immediately capped to prevent loss of oil due to volatilization. Salinity and temperature conditions were 30‰ S and 15°C, the laboratory optima for development (Sastry, 1970). Larvae were transferred to fresh seawater daily, survival monitored, and then they were fed day-old *Artemia salina* nauplii in excess. Development time was considered to be the number of days required from hatch to megalopa stage. Preliminary experiments on the effects of *A. salina* concentration on ingestion rates of Stage I zoeae showed that feeding is independent of food concentration at food levels between 4 and 30 *A. salina* ml^{-1} (Table 1). Subsequent experiments revealed that this relationship is constant throughout larval development (D. M. Johns, unpublished data). The food concentrations maintained in the rearing jars throughout the experiments were within these limits.

Ingestion rate, respiration rate, larval dry weight and ash content were determined for groups of larvae at each stage of development (5 zoeal stages and 1 megalopa). For Stage I zoea, all determinations were made between 24 and 36 h after hatching. For all other stages, the larvae used in experiments were between 24 and 36 h post-molt from the previous larval stage. The following sampling schedule was used: Stage II zoea, 6 days after hatching; Stage III zoea, 11 days; Stage IV zoea, 16 days; Stage V zoea, 21 days; and megalopa, 27 days after hatching.

Ingestion was measured by placing individual larvae in a 5 ml beaker containing 4 ml of filtered sea water with an initial food concentration of either 10 *Artemia salina* ml^{-1} (for zoeal stages) or 15 *A. salina* ml^{-1} (for the megalopa stage). Ingestion rates were measured as the difference between the number of *A. salina* initially offered to a larva and those remaining after a 24 h period.

Respiration rates were determined using all-glass differential microrespirometers (Grunbaum *et al.*, 1955). The number of larvae used in each determination varied with larval size, ranging from 5 larvae for Stage I zoea to 1 larva for Stage V zoea and megalopa. The respirometers were allowed to equilibrate in a water bath at 15°C for 15 min before initial readings were taken; subsequent readings were taken every 20 min for 2 h.

Dry-weight determinations were made with larvae from each respiratory study combined with others taken from the mass-rearing jars. Individuals were rinsed in deionized water, dried to a constant weight at 80°C and weighed on a Perkin-Elmer Autobalance[1] to the nearest 1.0 µg. Ash weights were determined by ashing previously dried larvae at 500°C for 24 h, and then reweighing. The dry weight and ash content of day-old *Artemia salina* nauplii were also determined.

[1]Reference to trade names does not imply endorsement by the United States Environmental Protection Agency.

Table 1. *Cancer irroratus*. Ingestion rates for Stage I crab zoea offered different densities of day-old *Artemia salina* nauplii

Initial *A. salina* nauplii density	Number of nauplii eaten zoea^{-1} 24 h^{-1}	*N*
3 ml^{-1}	9.33	7
5 ml^{-1}	8.00	7
10 ml^{-1}	8.20	7
20 ml^{-1}	9.13	7
30 ml^{-1}	8.68	7

One-way analysis of variance was used to test for significant differences in feeding rates and dry weights between control and oil-treated larvae. Larval growth, defined here as the increase in dry weight from one stage to the next, was fitted to the exponential growth model

$$Y = ae^{bX},$$

where Y is the weight of a larva and X is the stage of development. Respiration data for control and oil-treated larvae were fitted to the linear regression model

$$\log Y = b \log X + \log a,$$

where Y is the respiration rate of an individual (µl O_2 consumed h^{-1} larva^{-1}) and X is the larval dry weight in milligrams. Subsequently, the fitted regression lines were tested for equality of regression.

Results

Cumulative per cent survival to the megalopa for control *Cancer irroratus* larvae was 64%, and for oil-treated larvae 32% (Fig. 1). Survival rates in both groups were comparable through Day 5, after which mortality began to increase differentially in the oil-treated group. On Days 5 and 6, the larvae molt from Stage I zoea to Stage II, an event which is apparently sensitive to this oil treatment. Development time to megalopa was slightly shorter for control larvae ($\bar{x}$ = 26.4 days) than for the oil-treated larvae ($\bar{x}$ = 26.8 days), but these differences are not significant (Fig. 1).

Ingestion rates of newly-hatched *Artemia salina* nauplii by *Cancer irroratus* larvae increased during development for both the control and oil-treated groups (Fig. 2). The increased food consumption was greatest in the controls, ranging from an average of 9.8 *A. salina* larva^{-1} day^{-1} in the early stages of development to 44 *A. salina* larva^{-1} day^{-1} at the megalopa stage. In the oil-treated group, ingestion ranged from 9.2 *A. salina* larva^{-1} day^{-1} to a high of only 29.8 *A. salina* larva^{-1} day^{-1}. The feeding rates of controls were significantly higher ($P \leq .05$) in all stages after Zoeal Stage II (Fig. 2).

The increase in larval dry weight during development is tabulated in Table 2. Graphically, this increase is seen to be exponential for both control and oil-treated groups (Fig. 3). Although the

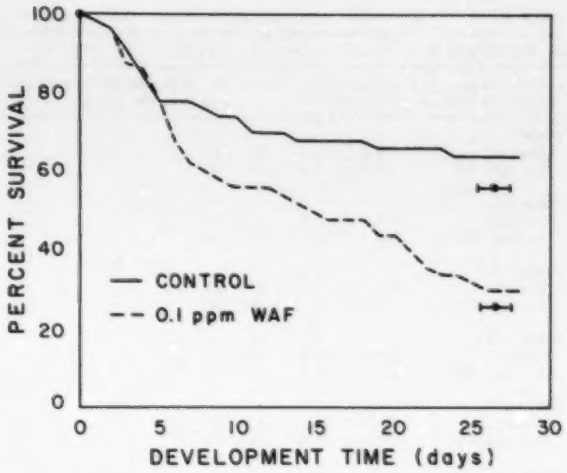

Fig. 1. *Cancer irroratus*. Survival of control and oil-treated crab larvae from hatching through metamorphosis to megalopae. Mean development time to megalopae ± 1 standard deviation is indicated by data points and bars on extreme right. N = 50. WAF: Water-accommodated fraction of No. 2 fuel oil

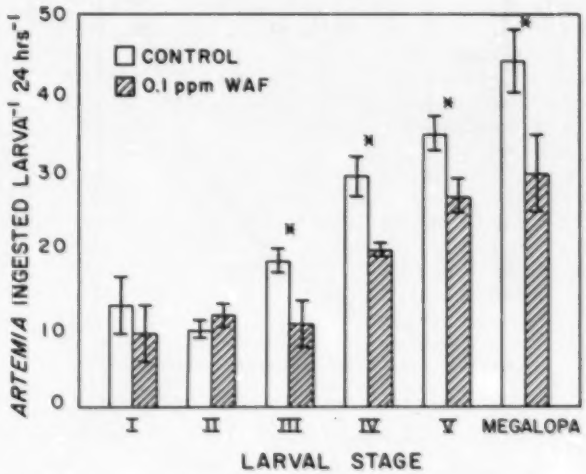

Fig. 2. *Cancer irroratus*. Mean ingestion rates of day-old *Artemia salina* by control and oil-treated crab larvae. Asterisks denote significant differences ($P \leq 0.05$) between control and oil-treated larvae. Error bars represent ± 1 SD

Table 2. *Cancer irroratus*. Growth of larval crabs reared in control and oil-treated conditions. Asterisks denote significant differences in dry weights between control and oil-treated larvae ($P \leq 0.05$)

Larval stage and rearing condition	Dry weight (μg larva^{-1})	N	% ash	% difference in dry weight between groups
Stage I				
Control	26.4 ± 2.0	(10)	27	0
0.1 ppm WAF	26.4 ± 2.0	(10)	26	
Stage II				
Control	41.0 ± 4.0	(7)	30	2.4
0.1 ppm WAF	40.0 ± 6.0	(7)	38	
Stage III*				
Control	84.0 ± 7.0	(7)	21	12
0.1 ppm WAF	75.0 ± 4.0	(8)	34	
Stage IV*				
Control	167.0 ± 10.0	(7)	24	43
0.1 ppm WAF	117.0 ± 8.0	(7)	30	
Stage V*				
Control	279.0 ± 43.0	(7)	21	59
0.1 ppm WAF	175.0 ± 14.0	(7)	19	
Megalopa*				
Control	709.0 ± 57.0	(7)	30	37
0.1 ppm WAF	517.0 ± 23.0	(7)	33	

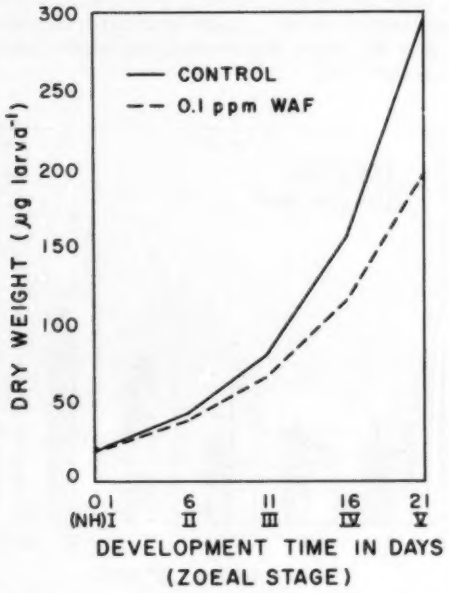

Fig. 3. *Cancer irroratus*. Larval growth, expressed as dry weight, during zoeal stages for both control and oil-treated crab larvae. Control $Y = 0.020\ e^{.129X}$; $r = 0.99$: oil-treated $Y = 0.020\ e^{.109X}$; $r = 0.98$

fitted curves are similar in shape, differences in the total amount of weight gained during development are apparent. Control larval weight increased 26 times from hatching to the megalopa stage; oil-treated individuals exhibited only an 18.6 fold weight increase in the same time period. There were no significant weight differences between the two groups for Stage I or Stage II larvae.

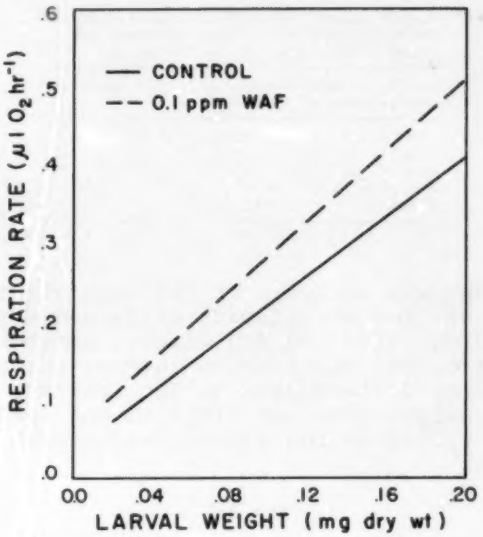

Fig. 4. *Cancer irroratus*. Respiratory rates of control and oil-treated crab larvae. Regression lines are significant from each other ($F = 5.85$, DF = 2,70) when tested for equality of regression: control, $\log Y = 0.807 \log X + \log 1.645$; $r = 0.89$; oil-treated, $\log Y = 0.822 \log X + \log 2.225$; $r = 0.89$

However, the weight differences are significant ($P \leq 0.05$) for all subsequent larval stages. The inorganic proportion of the dry weight is represented in Table 2 as percent ash. Generally, the oil-treated larvae exhibited higher percentages of inorganic matter than did the controls. Mean ash content for the controls was 25% dry weight (ranging from 21 to 30%) while ash content was 30% (ranging from 19 to 38%) for the oil-treated group (Table 2).

Routine respiratory rates of both groups were fitted to linear regression models (Fig. 4). Respiratory rates of the megalopa were omitted from the analysis due to the dramatic morphological, physiological, and behavioral changes that take place during and after metamorphosis from the zoea to the megalopa (Costlow and Sastry, 1966; Kalber and Costlow, 1966; Costlow, 1968). For control larvae, the regression coefficient $b = 0.80$, while for the oil-treated larvae, $b = 0.82$; however, these values are not significantly different from each other. Respiratory rate increased with increasing zoeal weight and, throughout the experimental period, oil-treated larvae consumed oxygen at a significantly greater rate ($F = 5.85$, DF = 2,70) according to an analysis of the equality of the two regression lines.

Discussion

Larval Survival and Development Rate

The 64% survival of control larvae from hatching to megalopae experienced in our study was within the laboratory culture survival range previously reported for *Cancer irroratus*; as was the development time from hatching to the megalopa stage (Sastry, 1970). While there was approximately a 50% reduction in survival attributable to oil exposure, there was no significant delay in development time. Many workers have found that environmental stress usually prolongs the intermolt period in brachyuran larvae (Costlow *et al.*, 1966; Katz, 1973; Bookhout *et al.*, 1976). The increased intermolt period usually results in increased development time as well as reduction in survival.

Mortality of oil-treated larvae in our experiments appears to be associated with the molting process. This was particularly apparent in the molt to Stage II zoea (Days 5-7, Fig. 1). Prior to the molt to Stage II, the mortality rates for the oil-treated larvae were similar to those of the controls. It was only upon molting that survival rates of the oil-treated larvae began to drop appreciably below that of the controls. Such increased mortality was also seen during the molt to Stage V zoea (Days 20-22). High mortality in oil-exposed crustaceans has also been reported during molting by Wells and Sprague (1976) for lobster larvae, *Homarus americanus*, treated with Venezuelan Tia Juana crude oil, and by Karinen and Rice (1974) for adult tanner crabs, *Chionoecetes bairdi*, also treated with crude oil.

Energetics of Larval Development

Unexposed, Control Larvae

Values of respiration rates, feeding rates, and growth reported here for *Cancer irroratus* larvae are within the range of values reported for other brachyuran larvae (Reeve, 1969; Belman and Childress, 1973; Mootz and Epifanio, 1974).

The relationship of respiratory rate to larval weight for *Cancer irroratus* larvae (b = 0.80) is somewhat higher than those reported for other crustacean larvae. The b values for other larvae range between 0.65 and 0.70 (Mootz and Epifanio, 1974; Logan and Epifanio, 1978), which fit the relationship of metabolic expenditure to spherical surface area more closely than does our value. All values however, fall within the range of b values (0.67 to 1.00) reported for most Crustacea (Wolvekamp and Waterman, 1960).

Increased feeding by the zoea during development has been noted for all other crustacean larvae thus far studied (Mootz and Epifanio, 1974; Logan and Epifanio, 1978). Control zoea consume up to 44 *Artemia salina* larva^{-1} day^{-1}, which represents a consumption of 26% of the body weight of a single crab larva. This value is comparable to the peak consumption rate of *Menippe mercenaria* zoea, which ingested up to 22% of their dry weight per day (Mootz and Epifanio, 1974).

Data collected on feeding rates and growth can be used to estimate gross growth efficiencies (growth ÷ ingestion x 100%; Welch, 1968) for the zoeal stages. We used ash-free dry weights in our calculations in order to measure only growth of organic tissue. The total number of *Artemia salina* consumed at each larval stage was estimated by multiplying the number of *A. salina* eaten per day at the beginning of the zoeal stage by the duration of the stage. This estimate assumes that the feeding rates of larvae within a particular stage remain constant during the intermolt period. It also assumes the zoea fully consumes the *A. salina* nauplii, although Dagg (1974) has reported that predatory crustaceans do not always consume the entire body contents of their prey.

Gross growth efficiencies for the zoeal stages of *Cancer irroratus* were between 6.2 and 39.6% (Table 3). This range of values compares favorably with values reported in the literature for other carnivorous crustacean larvae. Mootz and Epifanio (1974) reported gross growth efficiencies of 12 to 43% for larvae of *Menippe mercenaria*; Logan and Epifanio (1978) reported a range of 18 to 44% for *Homarus americanus* larvae. Reeve (1969) found a mean gross growth efficiency of between 50 and 60% for the larval stages of *Palaemon serratus*. The efficiencies reported by Reeve were calculated on a dry-weight basis rather than on an ash-free or caloric basis. It appears that his higher efficiency may be due to the inclusion of inorganic components in his calculations. Our gross growth efficiencies compare closely to Reeve's if total dry weight is used, with growth efficiency for Stage V zoea increasing from 39.6 to 58%.

The pattern of gross growth efficiencies suggests that the larvae convert food to tissue at similar efficiencies for the first four zoeal stages (Table 3). There is a dramatic increase in gross growth efficiencies in the last zoeal stage, a phenomenon also observed

Table 3. *Cancer irroratus*. Gross growth efficiencies for each zoeal stage reared in control and oil-treated conditions

Larval stage and rearing condition	Ash-free weight of food consumed (μg) (I)	Ash-free growth (μg) (G)	Gross growth efficiency (G/I x 100%)
24 h, post-hatch			
Control	51.2	6.4	12.5%
0.1 ppm WAF	36.8	6.6	17.9%
Stage I			
Control	256.0	15.8	6.2%
0.1 ppm WAF	184.0	11.9	6.5%
Stage II			
Control	196.0	37.5	19.0%
0.1 ppm WAF	232.0	24.7	10.6%
Stage III			
Control	372.0	60.4	16.2%
0.1 ppm WAF	208.0	32.5	15.6%
Stage IV			
Control	586.0	93.0	15.8%
0.1 ppm WAF	400.0	60.0	15.0%
Stage V			
Control	696.0	276.0	39.6%
0.1 ppm WAF	536.0	206.0	38.4%

in the terminal stage zoea of *Menippe mercenaria* by Mootz and Epifanio (1974). Costlow (1968) suggests that major reorganization and growth of tissue precedes metamorphosis into the megalopa stage. The higher gross growth efficiencies during this stage of development support this idea.

Effects of Oil Exposure

The water-accommodated fraction of No. 2 fuel oil substantially affected several physiological functions of the larvae. Food consumption and growth rates declined for oil-exposed larvae and metabolic expenditures for maintenance increased. Gross growth efficiencies were not altered by oil-exposure however.

A reduction in ingestion rates among organisms exposed to oil has been reported previously. Gilfillan (1975) found that adult *Mytilus edulis* and *Modiolus demissus* exposed to extracts of crude oil exhibited reduced filtration rates. Similar results have been found for larvae of *Crepidula fornicata* exposed to water-accommodated fractions of No. 2 fuel oil (J.A. Pechenik, unpublished data). Blumer *et al.* (1973) observed reduced responsiveness of oil-exposed lobsters and snails to food, which would result in reduced food consumption. This appears to be a general response of organisms exposed to a wide range of environmental stressors (Pandian, 1975).

An increase in metabolic rate (i.e., energy utilization) results in a reduction of energy available for growth if there are no compensatory changes in food consumption and/or assimilation efficiencies. There are no indications of compensation in either of these by *Cancer irroratus* larvae. As previously stated, oil exposure significantly reduces food intake and increases respiration rates. Assimilation efficiencies were not determined. However, the fact that oil-treated larvae grew less, clearly demonstrates an absence of effective compensatory changes.

This sublethal dose of No. 2 fuel oil (WAF) caused an increase in the rate of metabolic expenditure in the larvae of *Cancer irroratus*, but did not alter the basic allometric relationship between body weight and routine oxygen demands. Possibly, the increase in oxygen consumption is due to increased activity (as in an avoidance response) or is in response to the needs of metabolic pathways which detoxify oil and its metabolites (Corner *et al.*, 1973; Eldridge *et al.*, 1977). Increased metabolism may also be a general response to stress.

Some information is available on the effects that long-term oil exposures have on population growth and structure. Pechenik *et al.* (1980) observed reduced fecundity in several marine invertebrates following sublethal oil exposures, and Gillfillan and co-workers (1976, 1978) demonstrated lingering effects of oil spills on clean populations as indicated by suppressed food consumption and growth. Populations of bivalves in Cosco Bay, Maine, and Chedabucto Bay, Nova Scotia impacted with oil as long as 7 years ago (Chedabucto Bay), are still showing signs of energy budget disruptions. More work is needed to fully realize the impact of oil on energy-flow alterations in developing larvae and its consequences at the population level. Yet, it is plausible that the combination of reduced larval survival, reduced growth rates and reduced fecundity could lead to a reduction in the number of larvae available for recruitment in localities experiencing chronic oil discharge or long-term seepage following an oil spill.

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